HPLC

VARIAN, INC.

380-LC Series

ELSD FOOD ANALYSES APPLICATIONS BOOKLET

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Agilent Technologies

www.varianinc.com

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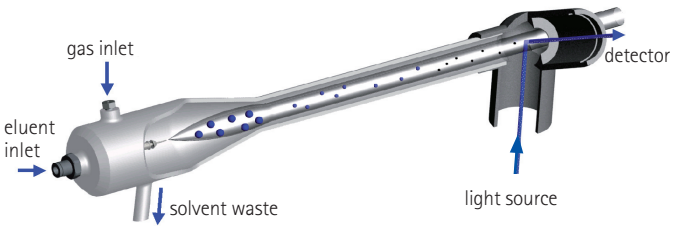
Universal Detection for Food Analysis

The Varian 385-LC is an advanced evaporative light scattering detector from Polymer Laboratories, now a part of Varian, Inc. The 385-LC delivers sub-ambient evaporation down to 10 °C, providing maximum sensitivity for compounds with significant volatility below ambient temperature. The instrument benefits from fast data output rates and extremely low dispersion for fast LC, and delivers a universal response down to the low-nanogram range for truly representative analysis. Reproducibility is less than 2 % for improved consistency of results. The Varian 385-LC offers real time gas management that eliminates solvent effects to give a constant response across a gradient. Control and digital data collection come as standard for multi-vendor platforms and so there is no need for an analog to digital converter. On-the-fly adjustment of light source intensity can save time during a run. Being complementary to LC/MS, and offering unrivalled flexibility and sensitivity, the Varian 380-LC series is the choice of ELSD for food applications.



Above is a general schematic of the Varian ELSD. Overall detector design is vital in ensuring the quality of detection and the level of reproducibility achieved.

Evaporative light scattering detection involves a three stage process.

1. Nebulization – utilizing an inert gas stream to form a plume of uniformly sized droplets.
2. Evaporation of the eluent – generating a plume of non-volatile solute particles.
3. Optical detection – where the intensity of scattered light is proportional to the mass of solute passing through the optical chamber.

Nebulization

Efficient nebulization using low gas flow rates is a feature of Varian ELS detectors. Independent nebulizer temperature control and digital gas flow control provide excellent stability and reproducibility. Baseline noise is minimized by the removal of any poorly nebulized eluent via a drain port.

Evaporation

The nebulized stream passes through an independently temperature-controlled evaporator tube where solvent is removed at high temperature, leaving the less volatile solute particles behind. The Varian 385-LC features patented[1] gas flow control technology with a short evaporator tube that gives an extremely low swept volume for minimal peak dispersion. This provides maximum resolution for high speed separations, especially important for work with small columns.

Optical Detection

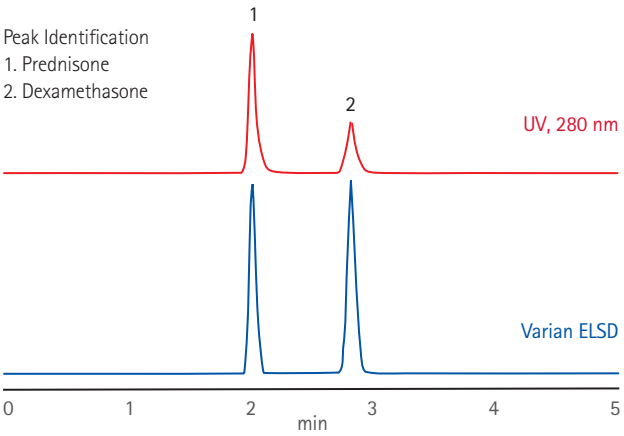
The solute particles are detected as they pass through the optical chamber. The high power LED and advanced design of the electronics delivers maximum sensitivity.

Benefits of ELS Detection

Obtain a more uniform response

The Varian 385-LC is not dependent on a compound's optical properties and so it provides a more uniform response than UV-Vis, making it the ideal detector for purity analysis or where calibration standards are not available.

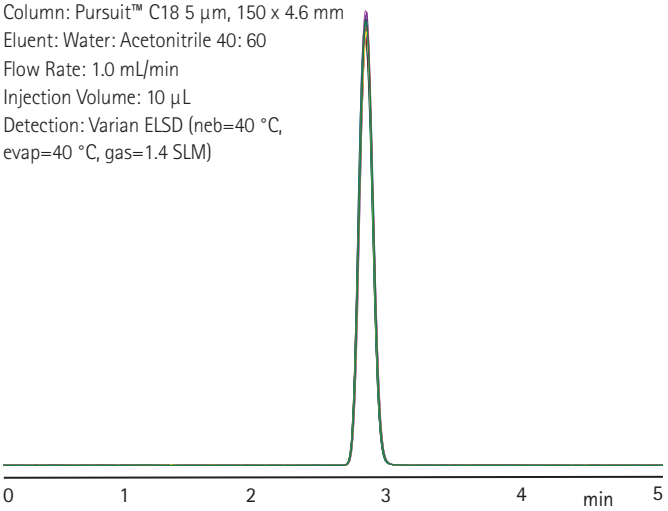
Column: Polaris™ C18 5 µm, 150 x 4.6 mm
Eluent: 50 % Water : 50 % ACN
Flow Rate: 1.0 mL/min
Injection Volume: 10 µL
Detection: Varian ELSD (neb=30 °C, evap=30 °C, gas=1.4 SLM); UV-Vis, 280 nm



Achieve superb RSD: 50 caffeine injections

Excellent reproducibility below 2 % gives reliable and accurate results - you can have complete confidence in your data.

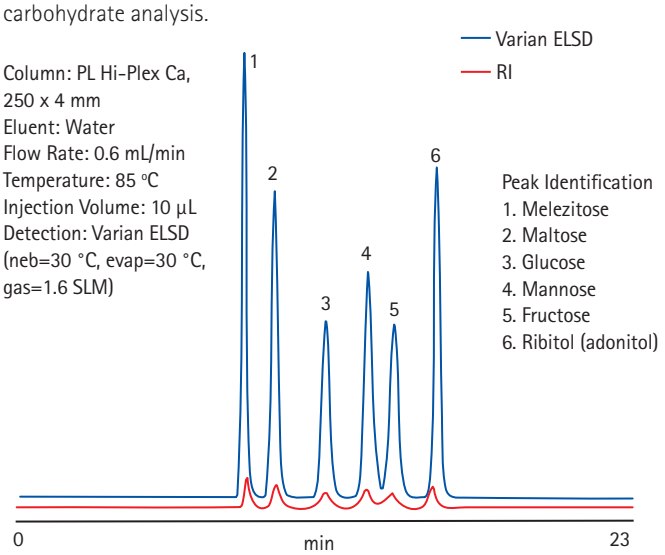
Column: Pursuit™ C18 5 µm, 150 x 4.6 mm
Eluent: Water: Acetonitrile 40: 60
Flow Rate: 1.0 mL/min
Injection Volume: 10 µL
Detection: Varian ELSD (neb=40 °C, evap=40 °C, gas=1.4 SLM)



Obtain higher sensitivity than with an RI detector

The Varian 380-LC ELSD has better baseline stability and sensitivity than an RI detector, making it an extremely suitable choice for carbohydrate analysis.

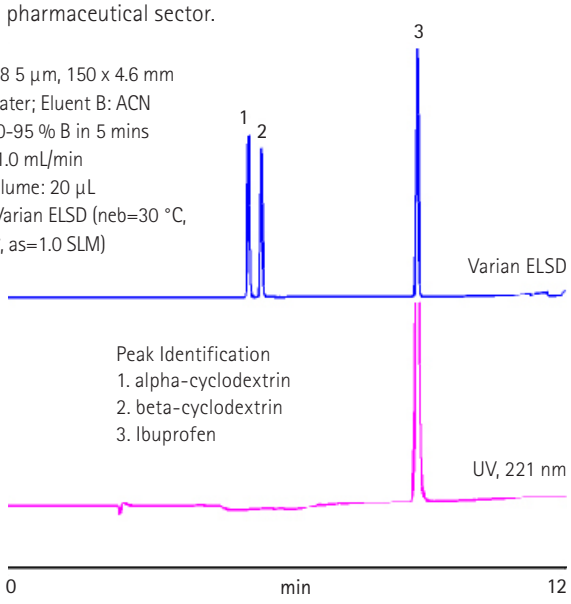
Column: PL Hi-Plex Ca, 250 x 4 mm
Eluent: Water
Flow Rate: 0.6 mL/min
Temperature: 85 °C
Injection Volume: 10 µL
Detection: Varian ELSD (neb=30 °C, evap=30 °C, gas=1.6 SLM)



Detect compounds with no UV chromophore

ELSD is necessary for compounds that do not possess a UV chromophore, but require gradient elution, such as cyclodextrins. Cyclodextrins are commonly used with hydrophobic drug molecules to improve the target compound's solubility, stability, bioavailability and dissolution. Consequently, their characterization is of great importance within the pharmaceutical sector.

Column: C18 5 µm, 150 x 4.6 mm
Eluent A: Water; Eluent B: ACN
Gradient: 50-95 % B in 5 mins
Flow Rate: 1.0 mL/min
Injection Volume: 20 µL
Detection: Varian ELSD (neb=30 °C, evap=50 °C, as=1.0 SLM)



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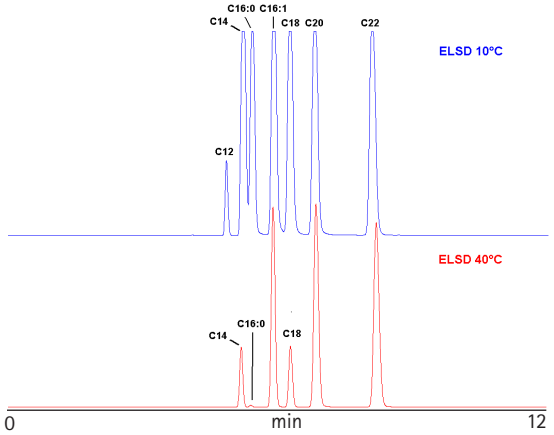
Lipids, Fats and Oils

Determine FAME using ELSD at 10 °C

Fatty acid methyl esters (FAME) are usually analyzed by GC/MS due to their high volatility. The FAMEs below C16 have typically been too volatile for ELSD, but the unique sub-ambient operation of the Varian 385-LC allows FAMEs to be analyzed at 10 °C, extending the detection range down to lauric acid methyl ester (C12).

Sample: Fatty Acid Methyl Esters mix (C8 to C22); Column: C18 5 µm, 250 x 4.0 mm; Eluent A: ACN; Eluent B: Dichloromethane; Isocratic: 75/25 A/B; Flow Rate: 1.0 mL/min; Injection Volume: 5 µL; Detection: Varian ELSD (neb=80 °C, gas=1.6 SLM)

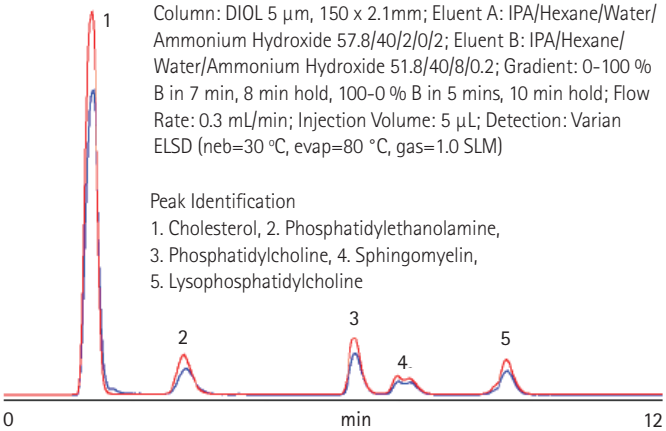


Detect phospholipids in complex matrices

Most polar lipids exhibit very poor UV chromophores and are typically derivatized to enhance their absorbance in the UV range. The use of RI detection is also not possible because complex gradients are required to attain the necessary resolution of phospholipid mixtures. The Varian ELSD provides universal detection that obviates the need for derivatization, allowing the rapid determination of lipids in complex matrices.

Column: DIOL 5 µm, 150 x 2.1mm; Eluent A: IPA/Hexane/Water/Ammonium Hydroxide 57.8/40/2/0/2; Eluent B: IPA/Hexane/Water/Ammonium Hydroxide 51.8/40/8/0.2; Gradient: 0-100 % B in 7 min, 8 min hold, 100-0 % B in 5 mins, 10 min hold; Flow Rate: 0.3 mL/min; Injection Volume: 5 µL; Detection: Varian ELSD (neb=30 °C, evap=80 °C, gas=1.0 SLM)

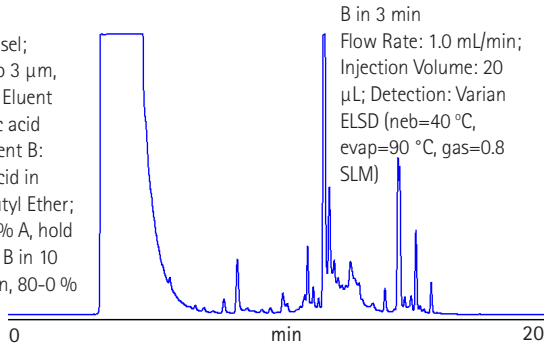
Peak Identification
1. Cholesterol, 2. Phosphatidylethanolamine,
3. Phosphatidylcholine, 4. Sphingomyelin,
5. Lysophosphatidylcholine



Detect compounds using complex gradient elution

Biodiesels can be produced from almost any vegetable oil, such as sunflower and rapeseed. They contain complex mixtures of fatty acid methyl esters and lipids, all of which possess weak or no UV chromophore. Consequently, fatty acids and lipids are often derivatized to enhance their UV absorbance or to facilitate their detection by LC/UV or GC/MS. ELSD removes the need to derivatize fatty acids and lipids, thus increasing sample throughput. Unlike UV and RI detection, ELSD is fully gradient compatible, which is a key advantage when analyzing biodiesels, where complex gradient elution is necessary to achieve the desired separation and resolution.

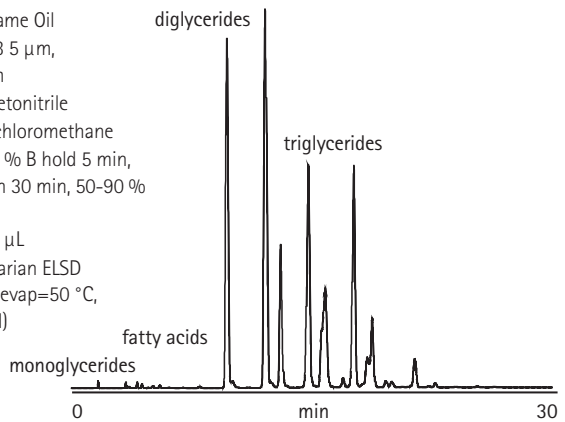
Sample: Biodiesel; Column: Cyano 3 µm, 250 x 4.6 mm; Eluent A: 0.4 % Acetic acid in Hexane; Eluent B: 0.4 % Acetic acid in Methyl tert-Butyl Ether; Gradient: 100 % A, hold 5 min, 0-80 % B in 10 min, hold 2 min, 80-0 % B in 3 min Flow Rate: 1.0 mL/min; Injection Volume: 20 µL; Detection: Varian ELSD (neb=40 °C, evap=90 °C, gas=0.8 SLM)



Achieve high sensitivity in triglycerides analysis

The composition of triglycerides in refined sesame oil using ELSD is outlined in Ph. Eur. monograph 0433. The oil contains many chemically similar species and so gradient elution involving solvents with very different properties is required to separate the components. For this reason, RI is not a viable method, while baseline disturbances can arise with UV or limit the choice of solvent. The Varian ELSD is unaffected by the optical properties of the solvents and delivers high sensitivity.

Sample: Sesame Oil
Column: C18 5 µm, 250 x 4.0mm
Eluent A: Acetonitrile
Eluent B: Dichloromethane
Gradient: 25 % B hold 5 min, 25-50 % B in 30 min, 50-90 % B in 5 min;
Flow Rate: 5 µL
Detection: Varian ELSD (neb=60 °C, evap=50 °C, gas=1.6 SLM)

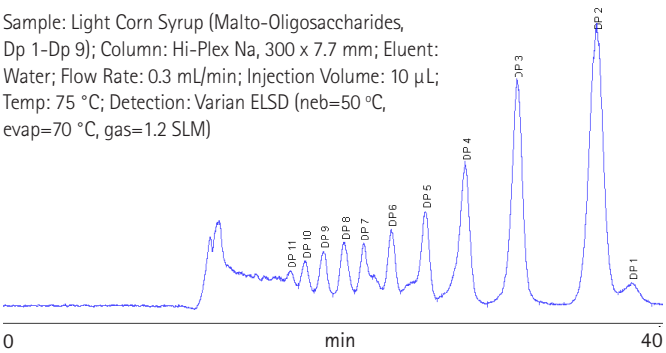


Carbohydrates

Achieve high sensitivity and stable baselines

Corn syrup is a common additive in food due to its thickening and humectant properties that keep foods moist and help to maintain freshness. It is also used to soften texture, add volume and prevent crystallization. The oligosaccharide composition of corn syrup, with its different degrees of polymerization (Dp), can affect sucrose crystallization in foods. The Varian ELSD is the ideal choice for determining the oligosaccharide composition of corn syrup, due to its high sensitivity and stable baseline, when compared to RI detection.

Sample: Light Corn Syrup (Malto-Oligosaccharides, Dp 1-Dp 9); Column: Hi-Plex Na, 300 x 7.7 mm; Eluent: Water; Flow Rate: 0.3 mL/min; Injection Volume: 10 µL; Temp: 75 °C; Detection: Varian ELSD (neb=50 °C, evap=70 °C, gas=1.2 SLM)

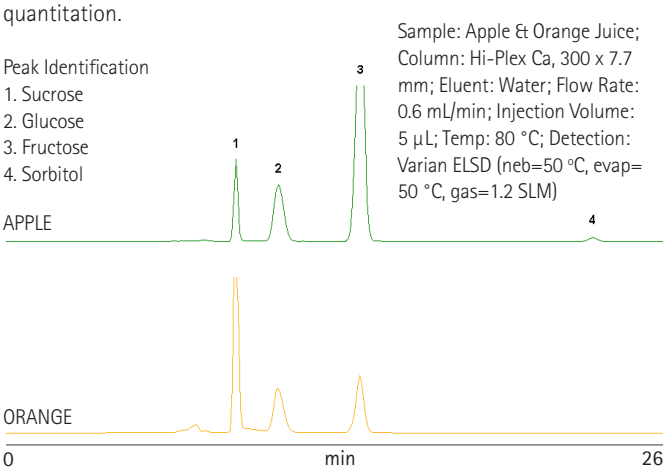


Improved detection of sugars in commercial fruit juices

The predominant sugars in natural fruit juice are sucrose, glucose and fructose. The ratio of these three sugars differs between fruit but with each single fruit the ratio is relatively constant. Fruit juices derived from concentrate are often adulterated with sweeteners, water or other types of fruit juices to extend the product life or to increase profit. The Varian ELSD is universal and can be used to directly measure the ratio of sugars in fruit juices. Compared to RI detection, the Varian ELSD provides a stable baseline, which improves the precision of the quantitation.

Peak Identification
1. Sucrose
2. Glucose
3. Fructose
4. Sorbitol

Sample: Apple & Orange Juice; Column: Hi-Plex Ca, 300 x 7.7 mm; Eluent: Water; Flow Rate: 0.6 mL/min; Injection Volume: 5 µL; Temp: 80 °C; Detection: Varian ELSD (neb=50 °C, evap= 50 °C, gas=1.2 SLM)

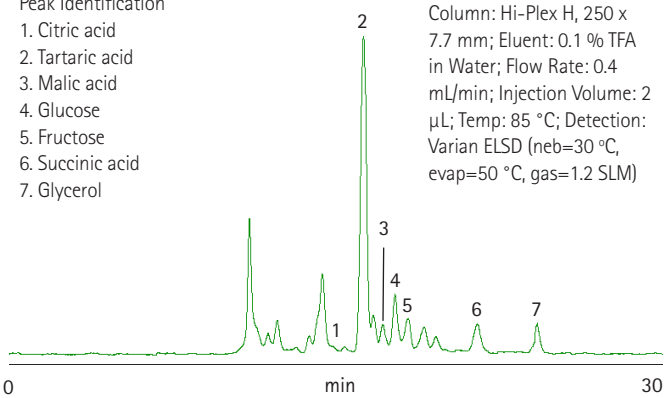


Measure organic acids & sugars in red wine accurately

Organic acids play an important role in the fermentation of wine. The precise balance of organic acids and sugars must be achieved in order to obtain the correct composition, stability and taste of the wine. The Varian ELSD is a universal and uniform detector that provides a simple and direct method of measuring the relative composition of organic acids and sugars in wine.

Peak Identification
1. Citric acid
2. Tartaric acid
3. Malic acid
4. Glucose
5. Fructose
6. Succinic acid
7. Glycerol

Sample: Australian Shiraz; Column: Hi-Plex H, 250 x 7.7 mm; Eluent: 0.1 % TFA in Water; Flow Rate: 0.4 mL/min; Injection Volume: 2 µL; Temp: 85 °C; Detection: Varian ELSD (neb=30 °C, evap=50 °C, gas=1.2 SLM)

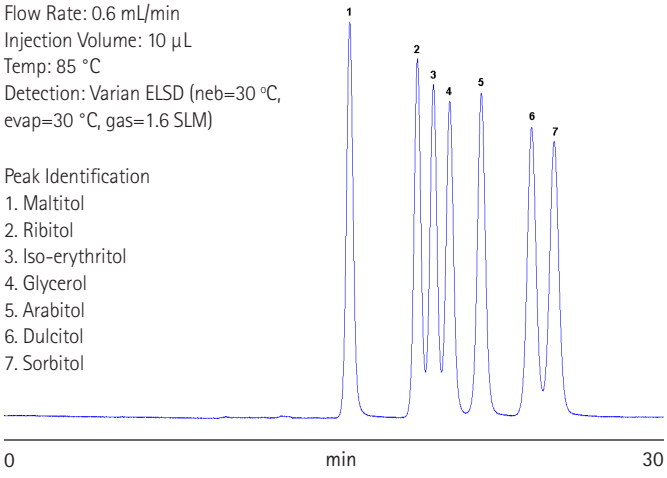


Detect sugar alcohols universally

Sugar alcohols are commonly used in place of sucrose, especially in diabetic foodstuffs. They also possess fewer calories than sucrose and do not contribute to tooth decay. The control of these additives in food can be carefully monitored using the Varian ELSD.

Sample: Sugar Alcohols
Column: Hi-Plex Ca, 250 x 4.0 mm
Eluent: Water
Flow Rate: 0.6 mL/min
Injection Volume: 10 µL
Temp: 85 °C
Detection: Varian ELSD (neb=30 °C, evap=30 °C, gas=1.6 SLM)

Peak Identification
1. Maltitol
2. Ribitol
3. Iso-erythritol
4. Glycerol
5. Arabitol
6. Dulcitol
7. Sorbitol

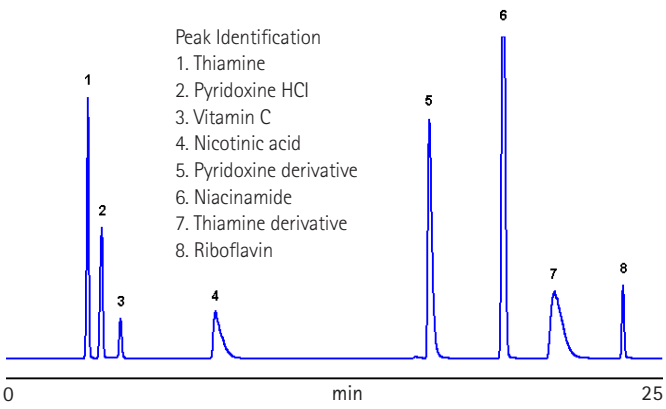


Vitamins and Food Dyes

Analyze accurate compositional data for vitamin mixtures

The universal and uniform response of the Varian ELSD provides accurate compositional data for vitamin mixtures. Highly polar vitamins, such as thiamine and vitamin C, can be problematic for UV detectors as they elute at the solvent front. The Varian ELSD evaporates the mobile phase before detection so does not exhibit any solvent front, which ensures that early eluting peaks are detected.

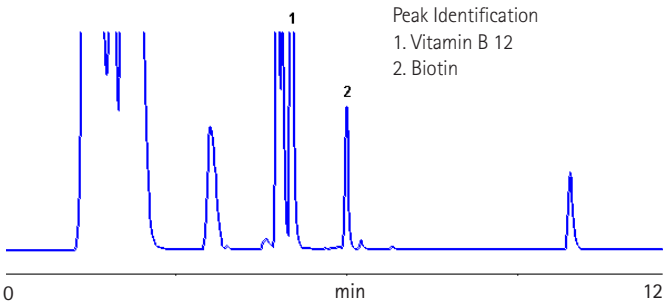
Sample: Water Soluble Vitamins mix; Column: C18 5 µm, 150 x 4.6 mm; Eluent A: Water, Eluent B: Acetonitrile; Gradient: 100 % A in 0-4 min, 0-50 % B in 10 min; Flow Rate: 0.6 mL/min; Injection Volume: 10 µL; Detection: Varian ELSD (neb=25 ˚C, evap=25 °C, gas=1.6 SLM)



Quantify vitamin B12 & biotin in nutritional supplements

The Varian ELSD provides direct quantitation of biotin and B12 without the need for derivatization. The universal response and high sensitivity of the ELSD allows biotin and vitamin B12 to be quantified in nutritional supplements.

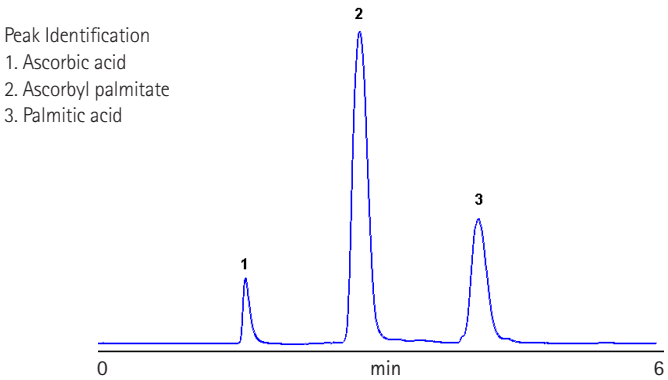
Sample: Nutritional Supplements; Column: PLRP-S 3 µm 100 Å, 50 x 4.6 mm; Eluent A: 0.1 % TFA in Water, Eluent B: 0.1 % TFA in ACN; Gradient: 1-99 % B in 10 min; Flow Rate: 0.5 mL/min; Injection Volume: 100 µL; Detection: Varian ELSD (neb=25 ˚C, evap=25 °C, gas=1.6 SLM)



Assess ascorbic acid and derivatives with accuracy

Ascorbyl palmitate is an ester formed from ascorbic acid and palmitic acid creating a fat-soluble form of vitamin C. It is commonly used as an antioxidant food additive (E304). The Varian ELSD can be used to accurately assess the relative ratios of these compounds due to its universal and uniform response.

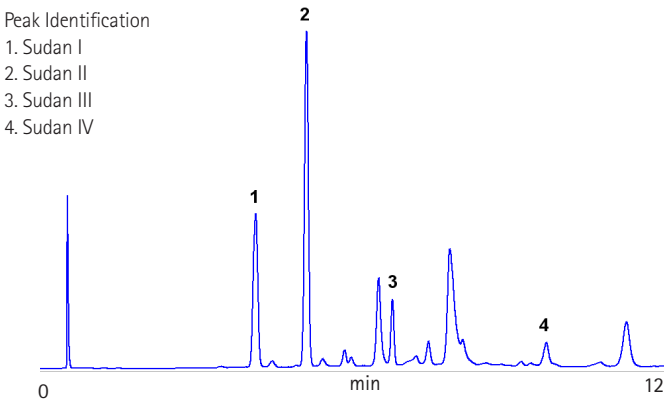
Column: C18 5 µm, 150 x 4.6 mm; Eluent A: 1 % Formic acid in Water, Eluent B: Acetonitrile, Eluent C: Methanol; Isocratic: 2.5/50/47.5 A/B/C; Flow Rate: 1.5 mL/min; Injection Volume: 10 µL; Detection: Varian ELSD (neb=30 ˚C, evap=30 °C, gas=1.4 SLM)



Determine sudan dyes in paprika oil

The Varian ELSD is universal and is not dependent on the optical properties of the compound. Consequently, for food containing Sudan dyes (I, II, III and IV) that possess UV different extinction coefficients, the ELSD delivers a more uniform and representative response.

Sample: Paprika Oleoresin; Column: C18 5 µm, 150 x 2.1 mm; Eluent A: 0.1 % Acetic acid in Water, Eluent B: 0.1 % Acetic acid in ACN; Gradient: 70-95 % B in 10 min, hold 10 min; Flow Rate: 0.23 mL/min; Injection Volume: 20 µL; Detection: Varian ELSD (neb=30 ˚C, evap=30 °C, gas=1.6 SLM)



Ordering Information

Product Description	Part Number
Varian 380-LC (240 V)	PL0890-0240
Varian 380-LC (110 V)	PL0890-0110
Varian 385-LC (240 V)	PL0890-1240
Varian 385-LC (110 V)	PL0890-1110
Varian ELSD Dimension Software	PL0890-0375
Varian ELSD Driver for ChemStation	PL0890-0380

Please refer to the publications below for additional application details.

Food Analyses	Application Note
Analysis of Complex Triglycerides in Starflower Oil from Borage by HPLC using ELSD	SI-01233 (TB1068)
High Resolution Analysis of Triglycerides in Vegetable Oils by HPLC with ELSD	SI-01231 (TB1066)
Rapid Identification of Illegal Dyes in Paprika Oil using HPLC with ELSD	SI-01237 (TB1072)
Sensitive Polar Lipid Analysis by HPLC using Low Temperature ELSD	SI-01224 (TB1058)
Rapid Profiling of Saccharides using HPLC with ELSD for Improved Precision	SI-01220 (TB1054)

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380-LC Series

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